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*Original*

Rapid determination of bacterial susceptibility to antibiotics combining Raman spectroscopy and Dielectrophoresis / Barzan, Giulia. - (2019). (Intervento presentato al convegno New frontiers for metrology: from biology and chemistry to quantum and data science tenutosi a Varenna, Como, Italy nel luglio 2019).

*Availability:*

This version is available at: 11583/2742002 since: 2019-07-15T12:03:14Z

*Publisher:*

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# Rapid determination of bacterial susceptibility to antibiotics combining Raman spectroscopy and Dielectrophoresis

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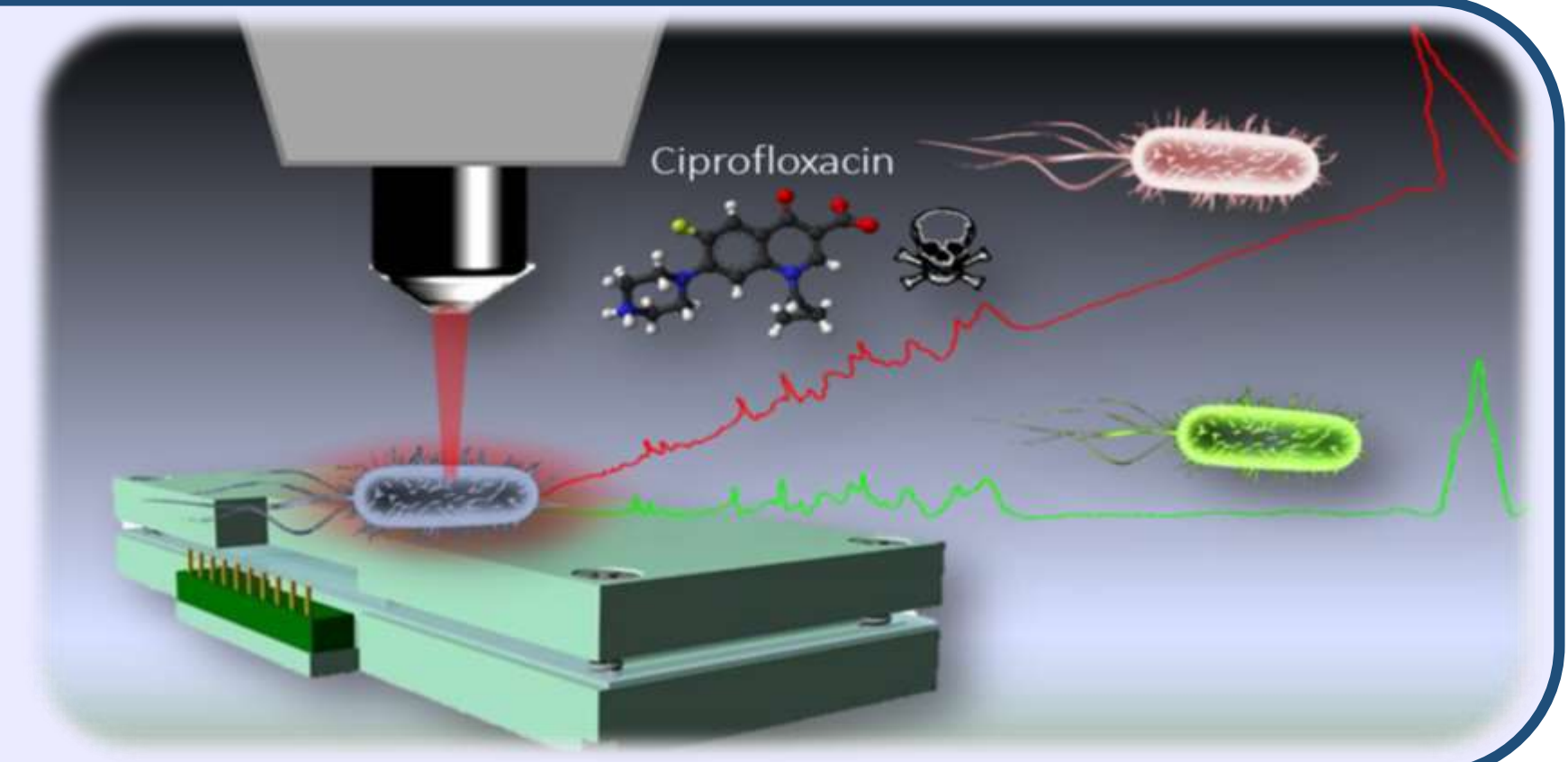
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## Introduction

The development of rapid, sensitive and specific methods to determine antibiotic susceptibility of bacteria is required to help reduce the widespread misuse of antibiotics and the growing multidrug-resistance problem [1]. This study presents a combined Raman spectroscopic and dielectrophoretic (DEP) approach to obtain direct, real-time measurements of a suspension of planktonic bacteria without the need of any labelling or other time-consuming sample preparation processes. Thanks to the spatial non-uniform DEP fields, bacteria are easily captured and concentrated under the laser spot of the Raman apparatus, increasing the sensitivity of the Raman technique [2]. Optimizing the setup conditions we were able to characterize different bacterial strains with high specificity. Using our Raman-DEP device, we demonstrated the susceptibility of *E. coli* towards the commonly prescribed second-generation fluoroquinolone [3] ciprofloxacin (CP), after only one hour of treatment, by monitoring spectral changes in the chemical fingerprint of the bacteria, which are related to the mode of action of the drug. Comparison between treated and untreated samples was performed at the MIC (minimum inhibitory concentration) and sub-MIC levels for different time points over a 3 hour span, and the Raman data were processed by supervised multivariate tools, such as PLS-Discriminant Analysis and PLS-Regression, for the calibration of descriptive models of cellular modifications. The models were validated using the cross-validation strategy. Simultaneously, standard microbiological assays based on cell viability, turbidity test and fluorescence microscopy, were carried out as reference methods to correlate the observed Raman response and to build strong predictive models.



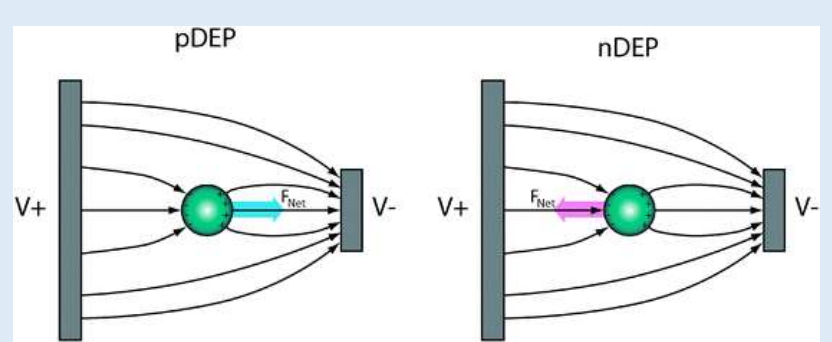
## The DEP device for Raman analysis of bacteria

### Dielectrophoresis (DEP)

Dielectric particles in a non-uniform electric field → **Net force**  
Magnitude of the force depending on permittivity of both particle and medium  
Can be positive or negative depending on which electrical permittivity is higher

Polarity of the electric field is irrelevant!

Variable (in time) electric field (AC)

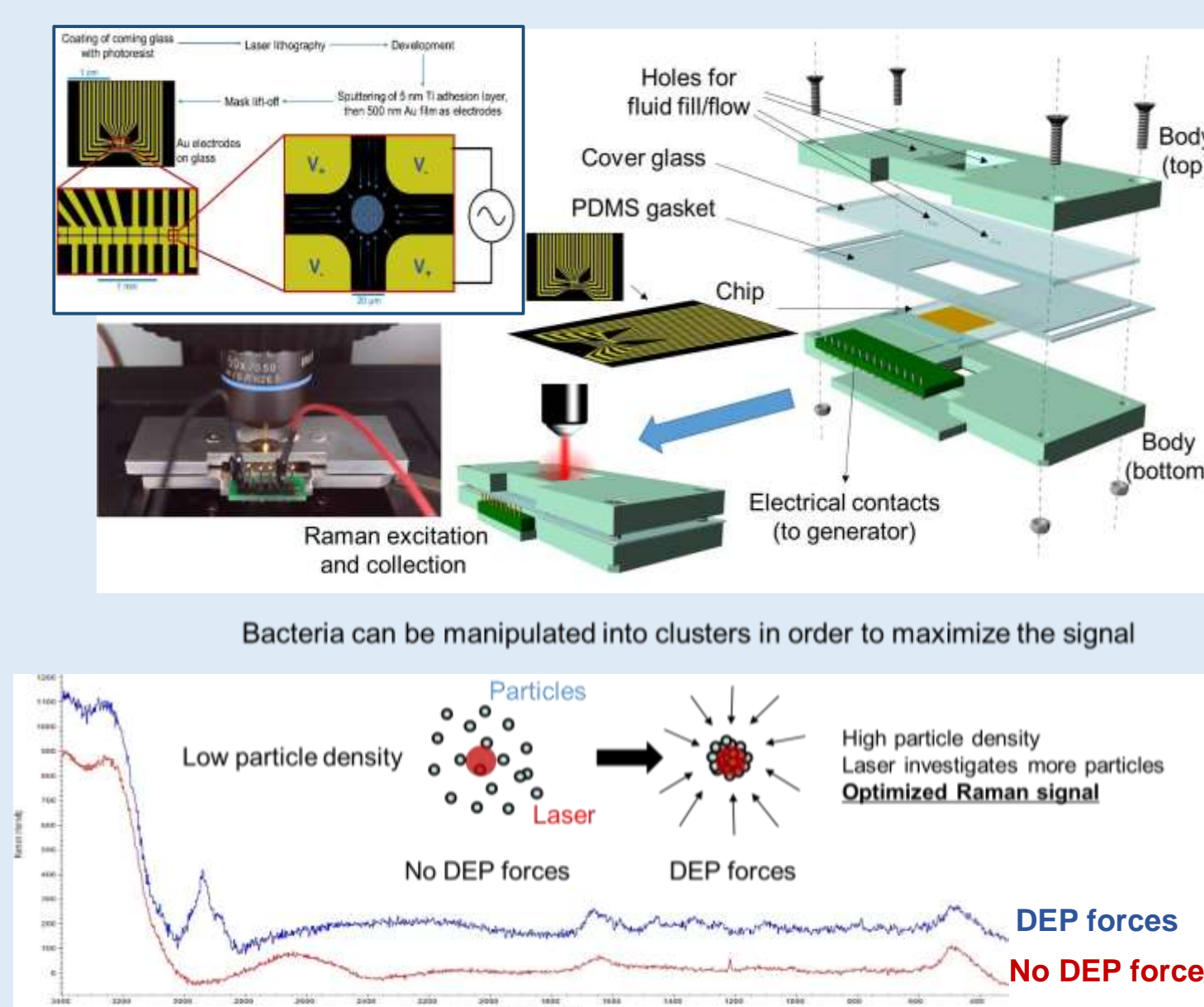


A dielectric particle placed in an electric field becomes electrically polarized as a result of partial charge separation, which leads to an induced dipole moment. The dipole moment is a consequence of the generation of equal and opposite charges at the boundary of the particle. In a non-uniform electric field, the particle experiences a net dielectrophoretic force. The magnitude of the induced dipole depends on the polarizability of the particle with respect to that of the medium. Applying the correct voltage and frequency, dielectric particles, such as bacteria, can be manipulated into clusters to increase their local concentration.

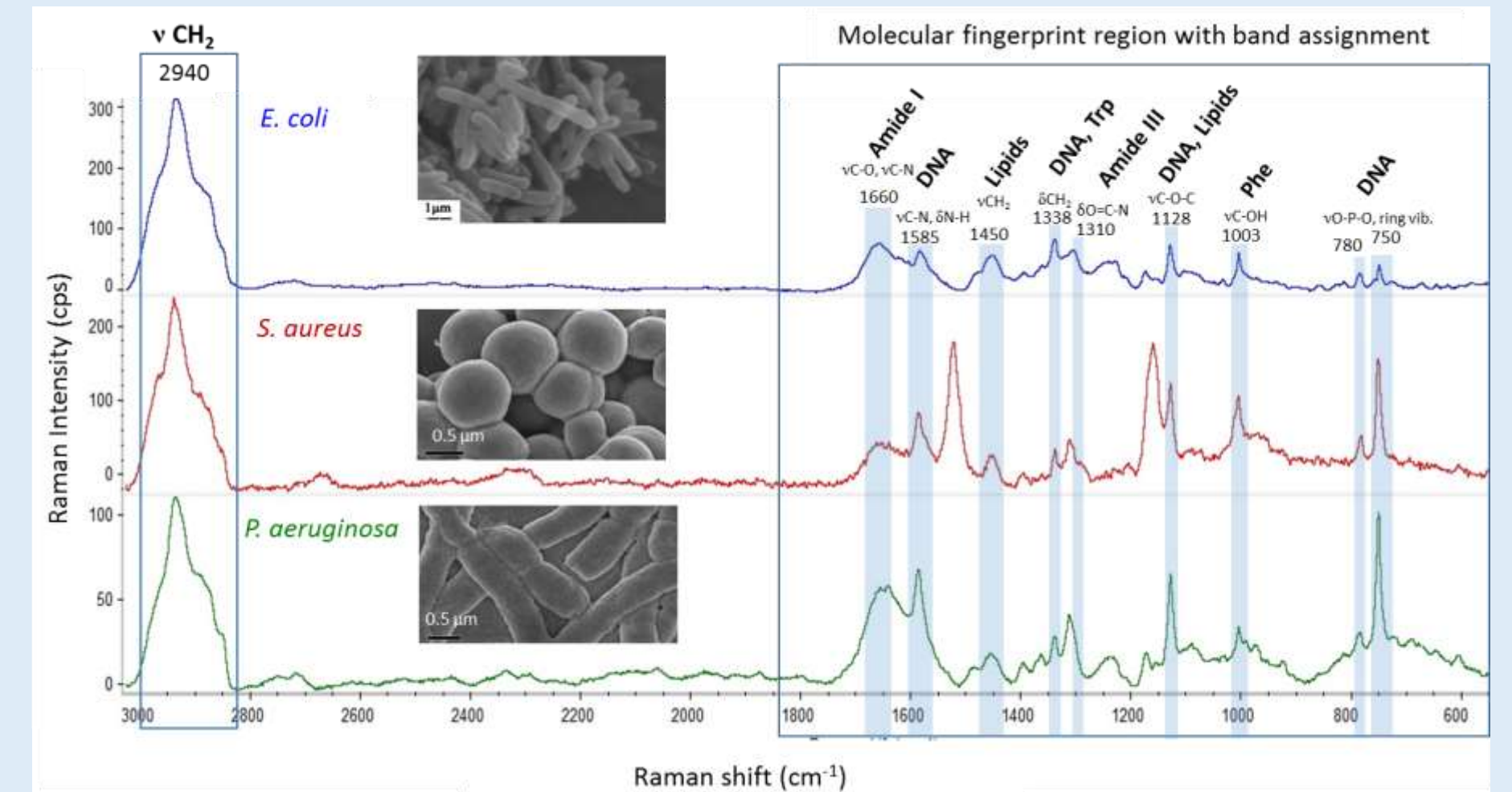
$$\langle \vec{F}_{DEP} \rangle \propto \text{Re} \left[ \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \right] |\nabla \vec{E}|^2$$

where  $\epsilon^* = \epsilon - j\frac{\sigma}{\omega}$  is the complex permittivity,  $\epsilon_p$  is the particle permittivity,  $\epsilon_m$  is the medium permittivity, and  $\omega$  is the frequency.

### Raman-DEP device



### Raman spectra of Bacteria



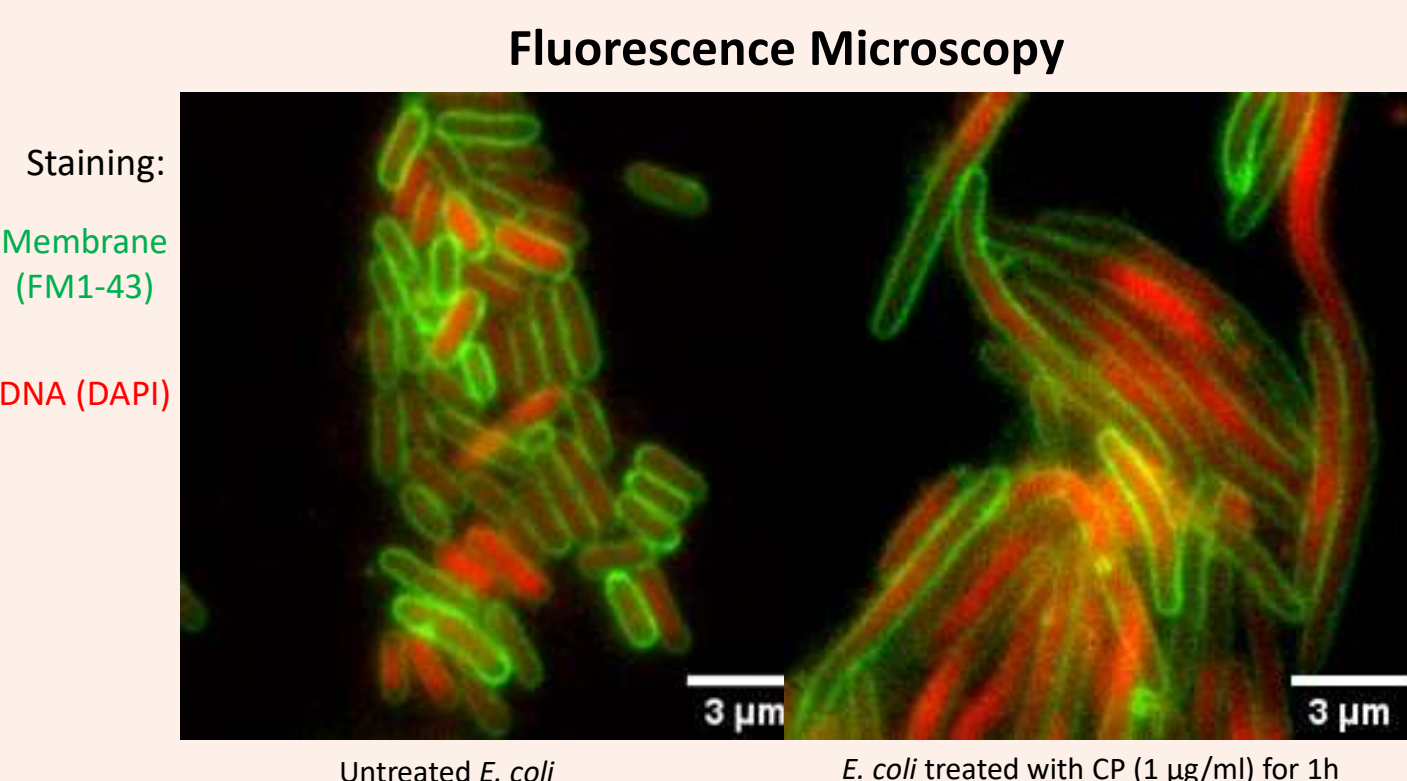
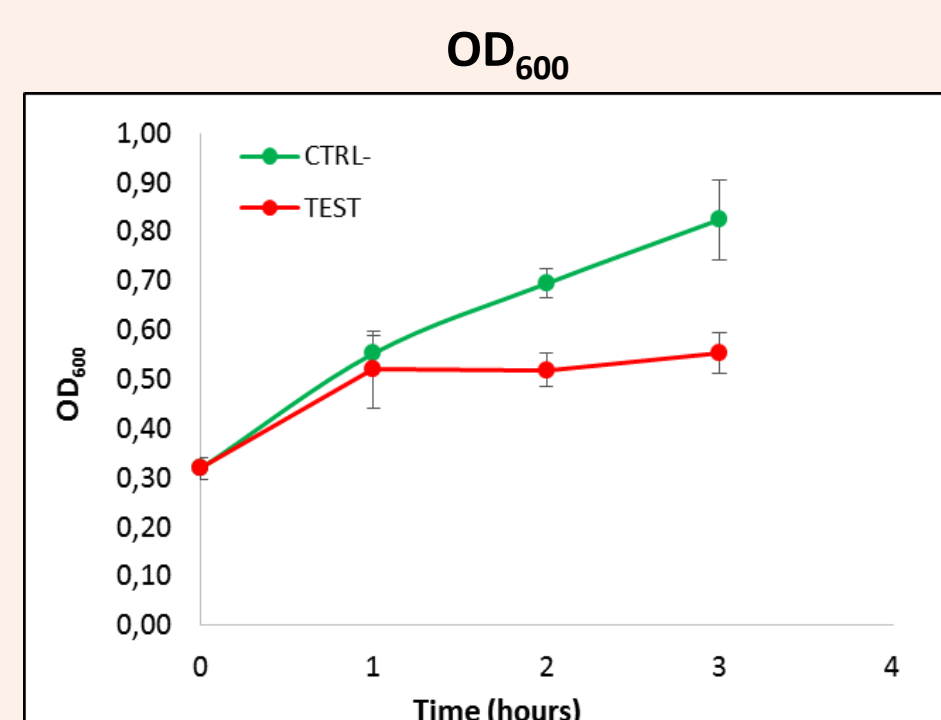
Raman characterization of: *E. coli*, *S. aureus* and *P. aeruginosa* using our DEP device. Optimized setup conditions for the cell: sinusoidal wave  $V = 5$  Vpp,  $f = 800$  kHz, aggregation time: 360 s. Optimized setup conditions for Raman microscope: laser line: 532 nm, laser power: 10 mW, objective: 60x water immersion, integration time: 2.5 s for 24 scans (1 min per spectrum).

## *E. coli* susceptibility to ciprofloxacin measured by Raman-DEP

### Calibration of the susceptibility test method

**Experimental setup:** Bacteria were treated with 1 µg/ml of ciprofloxacin in the middle of their exponential growth phase, when their  $OD_{600nm}$  was 0.3, during which they are more sensitive to the antibiotic.

MIC of CP in *E. coli* MG1655 ( $OD_{600nm} = 0.3$ ): **0.5 µg/ml**  
MBC of CP in *E. coli* MG1655 ( $OD_{600nm} = 0.3$ ): **1 µg/ml**

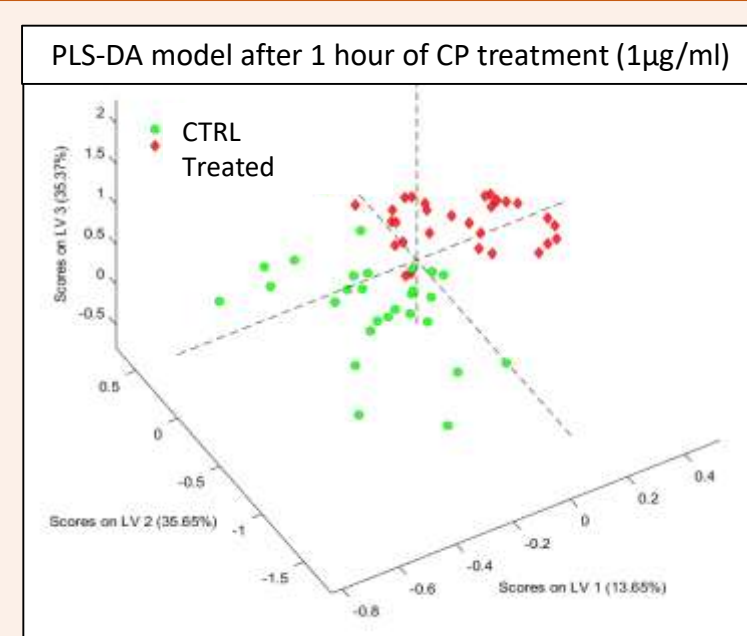


*E. coli* MG1655 untreated and treated with 1 µg/ml ciprofloxacin (CP) over a 3 hour span.

Morphological changes of *E. coli* due to the effects of the CP treatment are already visible after 1 hour.

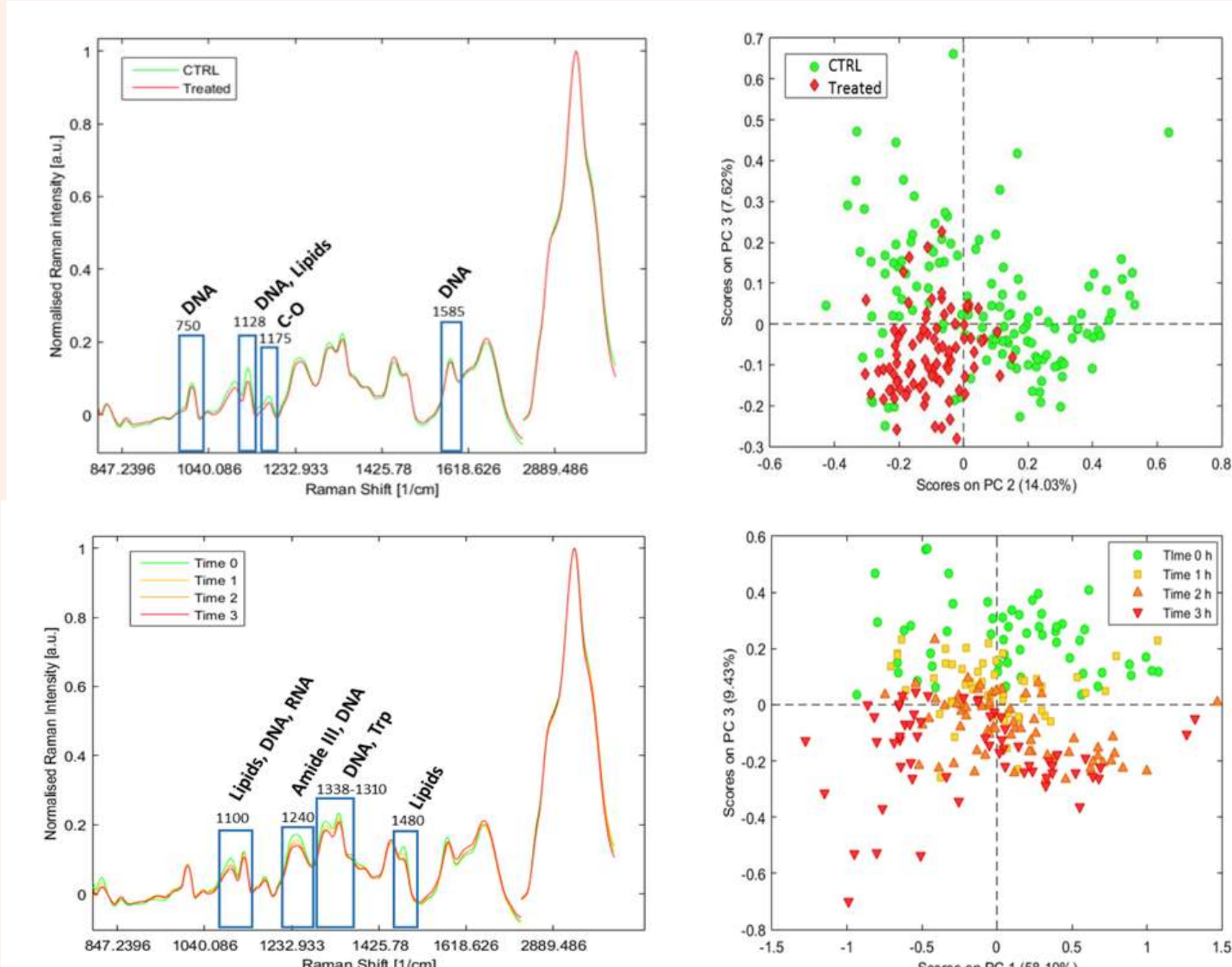
**PREDICTIVE CLASSIFICATION METHOD:**  
Partial least square-Discriminant Analysis (PLS-DA) model was used to classify the spectra of bacteria according to their response to antibiotic treatment.

	1 h	2 h	3 h
Sensitivity (Prediction)	0.67	1.00	1.00
Specificity (Prediction)	1.00	0.50	0.33
Class. Error (Prediction)	0.17	0.25	0.33



Six independent experiments were conducted for the construction of the model, five were used for the training and one for the validation.

### Raman spectra of CTRL and treated bacteria collected over time with the DEP device



Principal Components Analysis (PCA) helps in the visualization of non random variation in spectral data of CTRL and Treated bacteria. The spectra of *E. coli* treated with CP are grouped in the PCA scores plot.

PCA also revealed that Raman spectra captured changes related to the passage of time in both CTRL and Treated samples.

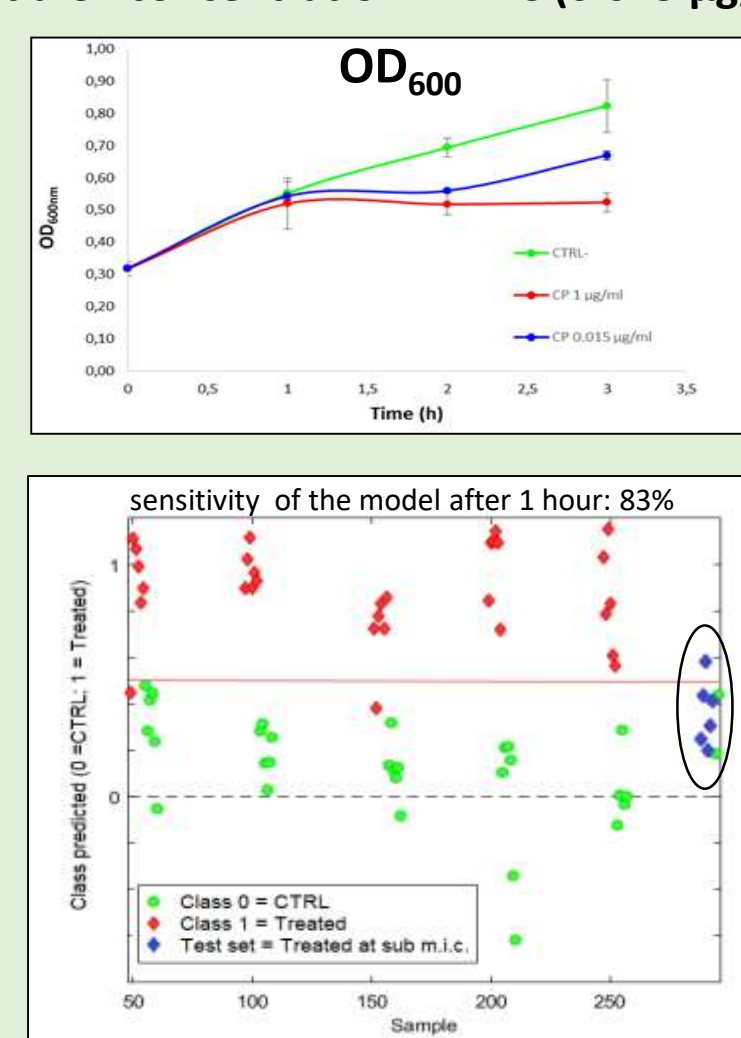
This effect was taken into consideration for subsequent classification analysis.

## Conclusions

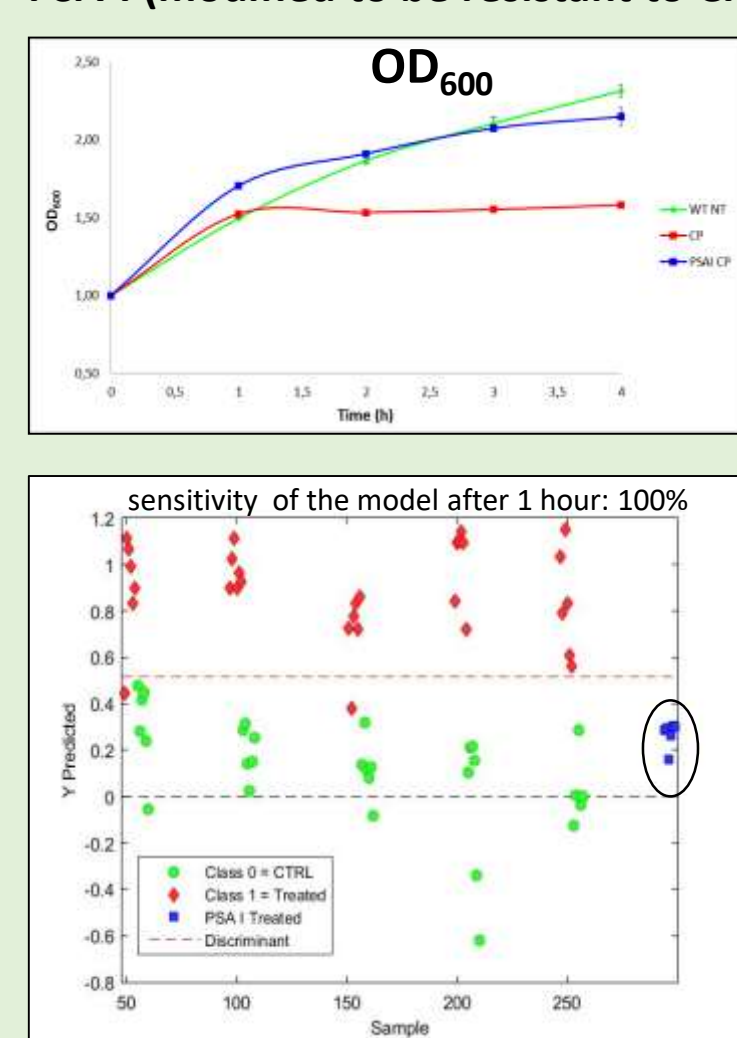
The Raman-DEP device here described allows to characterize different bacterial strains with high specificity and to follow dynamic interactions of the bacteria with antibiotics. Raman data processed with supervised multivariate data analysis are able to detect subtle spectral differences at a molecular level between treated or untreated bacterial cells after only 1 hour of treatment. This Raman-DEP method could open the way to rapid bacterial antibiotic susceptibility test without the necessity of time consuming sample preparation and overnight incubation required by classical microbiological techniques.

## Validations and applications of the method

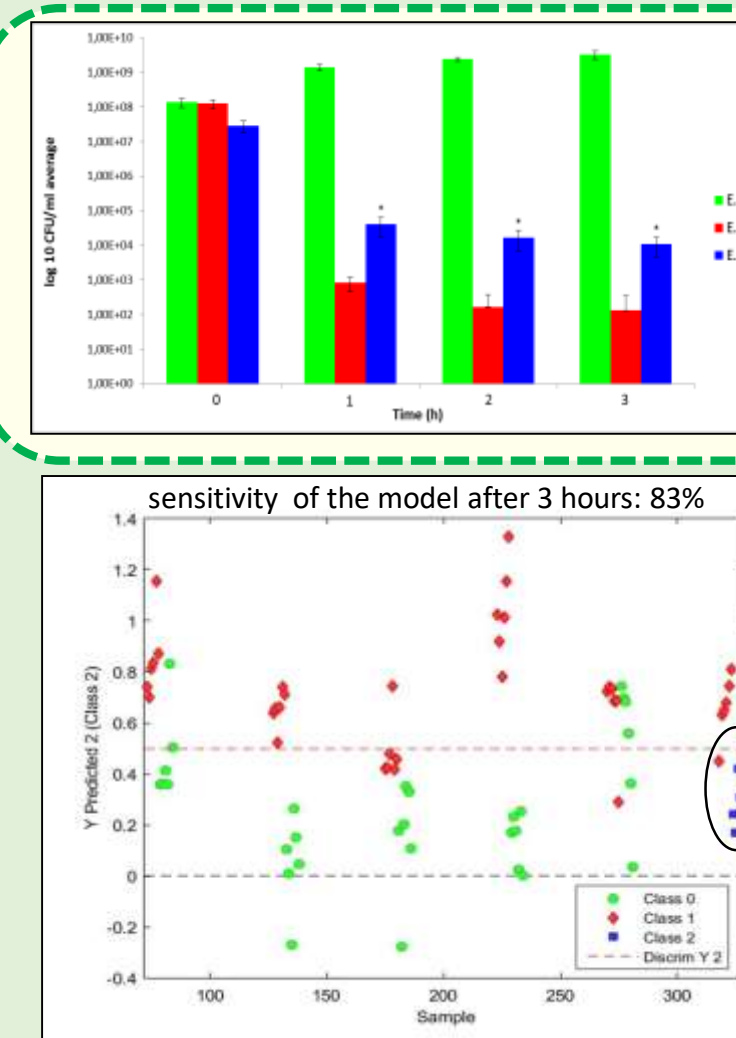
### 1. Prediction of the susceptibility of *E. coli* at a CP concentration <<MIC (0.015 µg/ml)



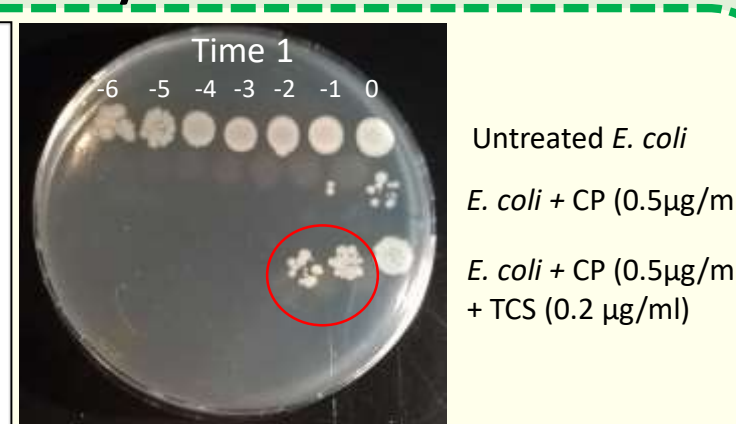
### 2. Prediction of the susceptibility of *E. coli* PSA-I (modified to be resistant to CP)



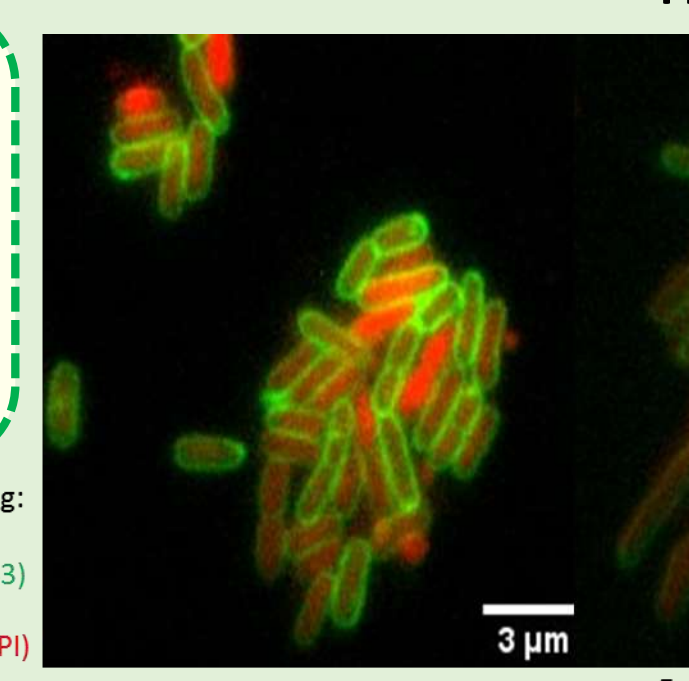
### 3. Prediction of the susceptibility of *E. coli* treated with CP at MIC (0.5 µg/ml) and triclosan (TCS) at sub-MIC (0.2 µg/ml), known to induce antibiotic resistance



### CFU assay



### Fluorescence Microscopy



Viability assay performed by counting the CFU (Colony Forming Units) and the fluorescence microscopy confirmed that bacteria pre-treated with triclosan at sub-MIC gained a sort of resistance towards the treatment with ciprofloxacin at MIC already after 1 hour.

Raman spectra of the *E. coli* treated with CP at sub-MIC or supposed to be resistant to the drug are all classified by the model as controls: viable bacteria, with high sensitivity.

## References

- [1] World-Health-Organisation in Antimicrobial resistance, Vol. 2014, 2014.
- [2] U.-C. Schröder et al., *J. Biophotonics* 10, 1547–1557, 2017.
- [3] O. Fasugba, A. Gardner, B. G. Mitchell, G. Mnatagazian, *BMC Infect Dis* 15, 2015.